6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, and/or measuring, and/or monitoring manganese, its metabolites, and other biomarkers of exposure and effect to inorganic and organic manganese compounds. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

Inorganic Manganese

Flame atomic absorption analysis is the most straightforward and widely used method for determining manganese (Tsalev 1983). In this method, a solution containing manganese is introduced into a flame, and the concentration of manganese is determined from the intensity of the color at 279.5 nm. Furnace atomic absorption analysis is often used for very low analyte levels (Baruthio et al. 1988), and inductively coupled plasma atomic emission analysis is frequently employed for multianalyte analyses that include manganese. Neutron activation analysis is also a very effective method for determining manganese concentrations in different samples (Kennedy et al. 1990; Rose et al. 1999). This technique uses no reagents and a minimum of sample handling; thus potential contamination with exogenous sources of manganese can be avoided. In addition, the technique has a low detection limit in biological tissues (4 ng/g) and high precision (Kennedy 1990). Further, the technique can be used for environmental samples as well as biological samples. Other methods for measuring manganese include spectrophotometry, mass spectrometry, neutron activation analysis, and X-ray fluorimetry.

It is important to note that none of these methods distinguish between different oxidation states of manganese or between different manganese compounds. Thus monitoring data on manganese is nearly always available only as total manganese present.

Organic Manganese

MMT. Levels of organometallic species in environmental and toxicological samples are typically in ppb concentrations, ng/mL in solution, or ng/g in solids (Walton et al. 1991). Therefore, methods of determination must be both selective and sensitive, achieved usually by coupling liquid or gas chromatography with detection via electrochemical, mass spectrometry, and atomic spectrometry detectors. A number of analytical methods for quantifying MMT in gasoline have been described including simple determination of total elemental manganese by atomic absorption and gas chromatography followed by flame-ionization detection. These methods usually measure MMT by detecting the metallic portion of the compound and reporting detection of MMT as manganese.

Maneb or mancozeb. The analysis of alkylenebis(dithiocarbamates) of some bivalent metal ions is hampered by their low solubility, low stability, and polymeric structure (Bardarov and Zaikov 1989). Furthermore, the methods developed for their determination have low selectivity. Indirect methods include spectrophotometric, gas chromatographic (GC), or thin-layer chromatographic (TLC) determinations of the reaction products, liberated after reduction (in an acidic medium) by carbon disulfide (Bardarov and Zaikov 1989). It is important to note that these methods are typically unable to distinguish among various dithiocarbamates since most can be degraded to CS_2. Other methods for determination of maneb or mancozeb, which are ethylenebisdithiocarbamates, or EBDCs, rely on the measurement of the metallic portion of the compounds, and therefore, many of these methods are similar to those for detection of inorganic manganese. Some newer methods are presented that have greater selectivity or use novel approaches different from carbon disulfide evolution.

6.1 BIOLOGICAL MATERIALS

Inorganic Manganese

Normally, determination of manganese in biological materials requires digestion of the organic matrix prior to analysis. For tissue samples or feces (detection limits ranging from 0.2 µg/g to <1 µg/g)), this is usually done by treatment with an oxidizing acid mixture such as 3:1:1 (v/v/v) nitric:perchloric:sulfuric acid mixture (Kneip and Crable 1988a). Fluid samples such as blood or urine may be digested in the same way (blood, detection limits = 1 µg/100 g, 10 µg/L), or manganese can be extracted by an ion exchange

resin (urine, detection limit = 0.5–2 µg/L) or by chelating agents such as cupferon in methylisobutylketone (urine, detection limit = <1 µg/L) . Recently, a method for directly measuring concentrations of trace elements in hair that does not require digestion prior to analysis has been developed (Stupar and Dolinsek 1996). While the authors used their technique to determine chromium, lead, and cadmium levels in hair, it is assumed that their slurry sampling or direct solid sampling technique might also work for manganese determination. Neutron activation analysis is also an effective analysis tool for measuring manganese in biological tissues (Rose et al. 1999, Vitarella et al. 2000). Table 6-1 summarizes some of the methods used for sample preparation and analysis of manganese in biological materials. It is important to note that special care is needed to avoid contamination of biological materials with exogenous manganese (Tsalev 1983; Versieck et al. 1988).

Organic Manganese

MMT. GC-FID may be used to determine levels of MMT in biological tissues and fluids with a detection limit of 1–2 ppm and percent recovery from 93.5 to 102.7% (Hanzlik et al. 1979).

More recently, Walton et al. (1991) have described high performance liquid chromatography coupled with laser-excited atomic fluorescence spectrometry (LEAFS) to detect various species of MMT. The detection limit for this GC-LEAFS method ranged from 8–20 pg of manganese for the various organomanganese species; the detection limit for determining manganese in MMT was 8 pg (0.4 ng/mL). This limit of detection was several orders of magnitude better than those for HPLC-UV or HPLC-AFC detection (Walton et al. 1991), but was worse than detection by GC-FID (DuPuis and Hill 1959). Walton et al. (1991) used their method to determine manganese species present in rat urine after rats had been administered MMT prepared in propylene glycol via subcutaneous injection.

Maneb or mancozeb. Headley (1996) used an inductively coupled plasma-atomic emission spectrometry (ICP-AES) method for occupational exposure estimations that measures mancozeb by determining the elemental manganese portion of the pesticide in a sample. The method was successful in analyzing urine (0.02 mg/L), wash water (0.02 mg/L), tank mixes (0.02 mg/L), cellulose acetate filters (0.5 µg), and fabric from patches and clothing (0.5 µg), with detection limits in parentheses. However, ICP-AES methods cannot differentiate among various forms of manganese, so it is important that background

Table 6-1. Analytical Methods for Determining Manganese in Biological Materials[a]

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	Extraction into methylisobutyl-ketone as the cupferon chelate	AAS (furnace technique)	<1 µg/L[b]	No data	Baselt 1988
Urine	Extract with resin, ash resin	ICP/AES	<1 µg/L[b]	100±10%	NIOSH 1984d
Blood	Acid digestion	ICP/AES	1 µg/dL	98±2.1%	Kneip and Crable 1988a
Blood	Digestion in oxidizing acid	ICP/AES	1 µg/100g	98±2.1%	NIOSH 1984c
Tissue	Digestion in oxidizing acid	ICP/AES	0.2 µg/g	98±2.1%	NIOSH 1984c
Tissue	Acid digestion	ICP/AES	0.2 µg/g	104±5.6%	Kneip and Crable 1988a
Feces	Dry at 110°, ash at 550°, dissolve in nitric acid	AAS (flame technique)	<1 µg/g	102±7%	Friedman et al. 1987
Hair	Digestion in concentrated nitric:perchloric acid (3:1) mixture	Flameless AAS	<0.2 µg/g	No data	Collipp et al. 1983
Hair	(a) slurry sample introduction technique (hair powder added to twice distilled water to measure bulk hair trace elements, or (b) direct introduction of hair segments to measure lontigudinal gradients	ETAAS (furnace technique)	No data	No data	Stupar and Dolinsek 1996[d]

Table 6-1. Analytical Methods for Determining Manganese in Biological Materials[a] *(continued)*

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Methods for determination of Maneb or Mancozeb					
Urine	Acid digestion in nitric acid or nitric acid-H_2O_2 mixture	Acid digestion in nitric acid or nitric acid-H_2O_2 mixture	0.020mg/L (based on 0.004 mg Mn/L) in urine	No data	Headley et al. 1996
Biological fluids and tissues	Extract into hexane containing biphenyl solutions containing 5-250 ppm MMT in 50 ppm biphenyl	Extract into hexane containing biphenyl solutions containing 5-250 ppm MMT in 50 ppm biphenyl	1-2 ppm	93.5-102.7%	Hanzlik et al. 1979
Tissues	Macerate autopsy tissues into fine slurry; Add anhydrous sodium sulphate, acetonitrile extraction with chloroform; Solution concentrated and residue dissolved in acetone	Macerate autopsy tissues into fine slurry; Add anhydrous sodium sulphate, acetonitrile extraction with chloroform; Solution concentrated and residue dissolved in acetone	approx. 0.5μg	90-95%	Tewari and Singh 1979

Table 6-1. Analytical Methods for Determining Manganese in Biological Materials[a] *(continued)*

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Methods for determination of MnDPDP					
Human plasma	Mix heparinized blood samples of patients receiving MnDPDP via injection with solid trisodium phosphate dodecahydrate pH 10.0±0.2; ultrafiltrate	Mixed-bed resin HPLC-Anion exchange and reverse-phase	0.8-2.3 µM (Mn cmpds) 0.1-0.8 µM (Zn cmpds) of 50 µL injection volume	85-115%	Toft et al. 1997a

[a]Magnetic resonance imaging (MRI) has been useful in determining brain accumulation of manganese but is not a quantitative method; therefore, it is not listed as an entry in this table.
[b]Estimated from sensitivity and linearity data
[c]Per sample size of 50 to 200 mL
[d]Methods were used to determine levels of chromium, lead, and cadmium in hair. Manganese concentrations in hair were evaluated for some, but not all, of the samples and tested one, but not both, new methods. However, it is assumed that both techniques will work for the trace element manganese.

AAS = atomic absorption spectroscopy; ICP/AES = inductively coupled plasma atomic emission spectroscopy; NIOSH = National Institute for Occupational Safety and Health; TLC = thin-layer chromatography

levels of manganese are known and low enough to not influence the levels of manganese attributable to mancozeb (Headley 1996).

Table 6-1 summarizes some common methods for the determination of manganese in various types of biological materials.

6.2 ENVIRONMENTAL SAMPLES

Inorganic Manganese

Manganese in air exists as particulate matter, so sampling is done by drawing air through a filter in order to collect the suspended particles. A variety of filter types (e.g., glass fibers and cellulose acetate) and sampling devices (e.g., low volume, high volume, and dichotomous) are available, depending on the particle sizes of concern and the concentration range of interest. In some cases, material on the filter may be analyzed directly (e.g., by X-ray fluorescence), or the filter may be digested by ashing in acid prior to analysis. In general, sensitivity is dependent on the volume of air drawn through the filter prior to analysis, and typically, detection limits are 1–2 µg/sample.

Water may either be analyzed directly, or, if the concentration of manganese is low, a concentration step (e.g., evaporation, extraction, and binding to a resin) may be employed (detection limits ranging from 0.005 µg/L–50 µg/L) . In all cases, acid is added to the sample to prevent precipitation of manganese. Using a new method, the catalytic kinetic method of analysis, Beklemishev et al. (1997) measured the concentrations of manganese in tap and river water. Their analytical method relies on an indicator reaction that is catalyzed by Mn(II) (the oxidation of 3,3',5,5'-tetramethylbenzidine [TMB] by potassium periodate [KIO_4]) and is carried out on the surface of a paper-based sorbent. The advantages of this new technique are that it has a much lower detection limit (0.005 µg/L) than do established methods and is transportable, allowing it to be used for rapid tests in the field (i.e., spot tests and similar procedures).

Determination of manganese levels in soils, sludges, or other solid wastes requires an acid extraction/digestion step prior to analysis. The details vary with the specific characteristics of the sample, but usually treatment will involve heating in nitric acid, oxidation with hydrogen peroxide, and filtration and/or centrifugation to remove insoluble matter.

Recently, manganese levels in foods have been determined in order to define more clearly human dietary requirements or levels of absorption of manganese from the diet (Tinggi et al. 1997). Atomic absorption spectrometry has been the most widely used analytical technique to determine manganese levels in a broad range of foods, as well as other environmental and biological samples (Tinggi et al. 1997). Tinggi et al. (1997) contributed a wet digestion technique using a 12:2 (v/v) nitric:sulfuric acid mixture for their determination, and, for food samples with low levels of manganese, they found that the more sensitive graphite furnace atomic absorption analysis was required. Because manganese is often found at very low levels in many foods, its measurement requires methods with similarly low detection limits; these researchers identified detection limits of 0.15 mg/kg (ppm) and 1.10 µg/kg (ppb) for flame and graphite furnace atomic absorption spectrometry, respectively (Tinggi et al. 1997). Neutron activation analysis is an effective technique for measuring manganese in environmental samples; it provides a low detection limit and high precision (Kennedy 1990).

Organic Manganese

MMT. A number of analytical methods for quantifying MMT in gasoline have been described including simple determination of total elemental manganese by atomic absorption (Smith and Palmby 1959) and gas chromatography followed by flame-ionization detection (FID) (DuPuis and Hill 1979). The former has measured manganese concentrations from 0.1 to 4 grams per gallon of gasoline after dilution of the sample with isooctane to minimize the effects of differences in base stock composition and is accurate to about 3% of the amount of manganese present. The latter has a detection limit of 1.7×10^{-14} g/s (0.017 pg/s) and could easily measure 6 mg/gallon of manganese in a gasoline sample; it is one of the most sensitive approaches. Aue et al. (1990) described a method in which MMT is detected in gasolines by gas chromatography coupled with flame photometric detection (FPD); the chemiluminescence of manganese is measured to determine MMT levels in a method that uses simple, inexpensive, and commercially available instrumentation. MMT levels can be determined down to 0.6 ppm (w/w) in gasoline (Aue et al. 1990). In another method showing excellent performance, Quimby et al. (1978) used gas chromatography followed by atmospheric pressure helium microwave detection system (or, microwave emission detector, MED); this method has a high degree of selectivity (1.9×10^{6}) and a detection limit of 0.25 pg/s at a wavelength of 257.6 nm.

Gas chromatography followed by electron-capture detection (ECD) (Gaind et al. 1992) or alternating current plasma (ACP) emission detection (Ombaba and Barry 1994) (detection limit: 62 pg as manganese) has also been described for determination of MMT in gasoline.

Gas chromatography followed by alternating current plasma (ACP) emission detection has been described for detecting MMT in air samples; airborne MMT concentrations as low as 0.001 mg/m^3 can be measured (Ombaba and Barry 1994).

Maneb or mancozeb. Like with many other dithiocarbamate pesticides, the most commonly used methods of detecting maneb or mancozeb involve degrading the active ingredient in the pesticide to carbon disulfide, CS_2. The CS_2 is then detected by spectrophotometry of a colored complex (Keppel et al. 1971) or by gas chromatography of the gas either in the headspace (McCleod and McCully 1969) or absorbed in a solvent layer (Headley 1996). The CS_2 evolution method is the AOAC method used widely to identify amounts of dithiocarbamates in pesticide formulations (HSDB 1999).

Examples of application of the carbon disulfide method include the determination of residues in or on food. McLeod and McCully (1969) developed a head space gas procedure for screening food samples (plants) for pesticide residues; while the method can quickly determine the presence of ferbam, maneb, nabam, thiram, zineb, and ziram in samples, it cannot differentiate among the dithiocarbamates or quantitate amounts of maneb. The CS_2 produced upon hydrolysis with hydrochloric acid-stannous chloride reagent is determined by gas chromatography; maneb recoveries ranged from 75–95% in samples of lettuce, cucumber, carrot, apple, cabbage, and strawberries. Alternatively, residues may be determined after reaction with acid to form carbon disulfide by measurement with gas liquid chromatography (Zielinski and Fishbein 1966) or standard colorimetric methods (HSDB 1999). Ahmad et al. (1996) describe an improved headspace gas-liquid chromatography (GLC) procedure used to measure dithiocarbamate residues in fruits and vegetables by detection of CS_2, followed by verification of EBDCs by conversion to ETU (ethylenethiourea), a degradation product. Although this method reduces false-positive results, it cannot differentiate among various dithiocarbamate pesticides and also may overestimate the apparent CS_2 content (Ahmad et al. 1996).

Rao et al. (1993) present a modification of the usual CS_2 evolution method (which measures CS_2 by spectrophotometry) with a method that converts maneb to a manganese-PAN complex that is extracted in

isobutyl methyl ketone (MIBK); the complex then absorbs at 550 nm and can be measured from 0.37 to 3.75 micrograms/mL. Their method determines micro-quantities of maneb in commercial formulations, synthetic mixtures, grain, and in the presence of various other dithiocarbamates. The authors note that this method is particularly selective since other pesticides like ziram, zineb, and ferbam which usually interfere in other methods did not interfere under their experimental conditions.

Hylin et al. (1978) present an ultraviolet absorption method for analysis of maneb formulations. The maneb is converted to nabam, a water soluble ethylenebisdithiocarbamate, by treatment with Na_4EDTA. The converted maneb is measured at 284 nm, and a conversion formula is given to calculate the % maneb.

Newsome (1974) presents a method in which, following hydrolysis with hydrochloric acid containing stannous chloride, maneb is converted to an ethylenediamine that is recovered on an ion-exchange column and determined by gas chromatography as the bis-trifluoroacetate (IARC 1976). The limit of detection was 0.1 mg/kg.

Walash et al. (1993) developed a spectrophotometric method for determination of maneb and its decomposition product, ETU, in some vegetables by using EDTA as a solvent which causes release of the EBDC moiety from maneb followed by reaction of EBDC with either 2,6-dibromoquinone chlorimide (DBQ) or 2,6-dichloroquinone chlorimide (DCQ). The result is a red solution that absorbs at 495 nm. Amounts as low as 2 ppm were detected in cucumber and tomato fruits.

Noguer and Marty (1997) propose a new high sensitivity biosensor method that allows the detection of dithiocarbamate fungicides at concentrations less than 10 ppb. By using the fact that these compounds strongly inhibit yeast aldehyde dehydrogenase (AIDH), they are trying to develop an amperometric bienzymic sensor for dithiocarbamate detection based on AIDH inhibition that determines the concentration of dithiocarbamates by measuring changes in current; while the method's operational stability is still in need of improvement, the method was able to measure 1.48 ppb maneb.

Rangaswamy and Vijayashankar (1975) describe a method that relies upon the periodate oxidation of the manganese in mancozeb to permanganic acid in the presence of phosphoric acid; this method can determine the active ingredient in formulations and can identify residues.

Afsar et al. (1987) have developed a method to differentiate mancozeb from a mixture of maneb and zinc salts or from a mixture of maneb and zineb. Compounds are distinguished on the basis of color differences after treatment of the saturated solutions of fungicides in *n*-propanol-acetone mixture first with dithizone and then with monosodium dihydrogen phosphate. Stevenson (1972) presented a similar earlier method that distinguished maneb, zineb, mancozeb, and selected fungicidal mixtures by successive application of acid dithizone, sodium hydroxide, and acid dithizone to the spot.

Spot tests for the in-field detection of mancozeb in water use copper (II) chloride-acetic acid as a coloring reagent. A cupric salt is formed which is soluble in chloroform extractant to produce a red-brown solution; this method has a detection limit of 15 µg (Rathore et al. 1996).

Because maneb and mancozeb have vapor pressures of virtually zero, they may be present in air only as dust (Maini and Boni 1986). Workroom air sampling has been performed by collection of maneb or mancozeb on filters followed by hydrochloric acid hydrolysis, with stannous chloride reduction, and analysis of the resulting carbon disulfide using gas chromatography (Maini and Boni 1986; Woodrow et al. 1995).

Table 6-2 summarizes some common methods for the determination of manganese in various types of environmental media.

6.3 ADEQUACY OF THE DATABASE

Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of manganese is available. Where adequate information is not available, ATSDR, in conjunction with the NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of manganese.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean

Table 6-2. Analytical Methods for Determining Manganese in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collection on filter, direct analysis	XRF	2 µg/sample	No data	NIOSH 1984a
Air	Collection on filter, acid digestion	ICP/AES	1 µg/sample (5 µg/m^3)	84-93%	NIOSH 1984b
Water	Acidify with nitric acid	AAS (furnace technique)	0.2 µg/L	No data	EPA 1983b
Water	Acidify with nitric acid	AAS (flame) AAS (furnace) ICP/AES	2 µg/L 0.01 µg/L 1 µg/L	No data No data No data	Taylor 1982
Water	Acidify with nitric acid	AAS (direct aspiration)	10 µg/L	100±6%	APHA 1985a
Water	Adjust pH to 2-4, extract with APDC into MIBK	AAS (direct aspiration)	<10 µg/L	No data	APHA 1985b
Water	Acidify with nitric acid	AAS (furnace technique)	0.2 µg/L	No data	APHA 1985c
Water	Acidify with nitric acid	ICP/AES	2µg/L	No data	APHA 1985d
Water	Acidify with nitric acid	AAS (direct aspiration)	10 µg/L	100±2%[a]	EPA 1983a
Water	Acidify, oxidize	Colormetric	50 µg/L	100±26%	APHA 1985e
Water	Preconcentrate manganese-containing solution and 3,3',5,5'-tetramethylbenzidine (TMB) onto filter paper; add oxidant KIO_4 to catalyze oxidation; measure absorbance	Catalytic kinetic method of analysis	0.005 µg/L	No data	Beklemishev et al. 1997

Table 6-2. Analytical Methods for Determining Manganese in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water and wastes	Acid digestion	ICP/AES	2 µg/L	100±6%	EPA 1982
Water and wastes	Acid digestion	AAS	10 µg/L	100±2%	EPA 1986c
Water and wastes	Acid digestion	ICP/AES	2 µg/L	93±6%	EPA 1986b
Sediments, sludges, soils	Acid digestion, oxidation, filtration/centrifugation	AAS, ICP/AES	Variable, depending on matrix	93±6%	EPA 1986a, 1986b
Foods	Digest wet or dry foods with HNO_3–H_2SO_4 mixture (12:2 mL)	AAS [flame(F) or graphite furnace(GF)]	F-AAS: 0.15 mg/kg GF-AAS: 1.10 μg/kg	No data	Tinggi et al. 1997
Methods for MMT Determination					
Air	Draw known volume of air through XAD-2 sampling tubes for 10-60 minutes	GC-ECD	0.001 mg/m^3 (in 10 L sample); 0.02 ng from a 2.0 µL injection of a 0.01 µg/mL MMT solution	No data	Gaind et al. 1992

Table 6-2. Analytical Methods for Determining Manganese in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Gasoline	Dilute gasoline in acetone (1:10)	Capillary GC-ACP detector	62 pg/s	No data	Ombaba and Barry 1994
Gasoline	Dilute with hexane (1:99); direct injection	GC-ECD	No data	No data	Gaind et al. 1992
Gasoline	Inject sample	GC-MED	0.25 pg/s	No data	Quimby et al. 1978
Gasoline	Inject sample	GC-FPD	0.6 ppm	No data	Aue et al. 1990
Methods for Determination of Maneb or Mancozeb					
Air	Trap mancozeb on glass fiber filters at 14-16 L/min; hydrochloric acid hydrolysis with stannous chloride reduction	Sulfur-mode flame photometric gas chromatography	0.5 μg/filter = 23 ng/m^3	No data	Woodrow et al. 1995
Water	Heat water sample in presence of tin(II) chloride and 2,2,4-trimethylpentane (isooctane); CS_2 dissolves in isooctane Note: result corresponds to total of dithiocarbamates that undergo this reaction	GC-FPD	0.84 μg/L as maneb	No data	Barceló 1993

Table 6-2. Analytical Methods for Determining Manganese in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Solution prepared in NaOH, copper (II) chloride-acetic acid used as coloring agent	Visual spot test	15 μg (may be lowered by preconcentration via column chromatographyo r liquid-liquid extraction	No data	Rathore et al. 1996
Maneb formulations	Treat with Na_4EDTA to convert maneb to nabam, a water soluble ethylenebisdithiocarbamate	Ultraviolet absorption: Measure converted maneb at 284 nm	No data	No data	Hylin et al. 1978
Commercial formulations, Grain, Dithiocarbamate mixture	Combine maneb with PAN at pH 9.2; Extract Mn-PAN complex in isobutyl methyl ketone (MIBK)	Spectrophotometry	0.37 µg/mL-3.75 µg/mL	97.5-98.5% (grain); 99.0-100.6% (synthetic mixtures)	Rao et al. 1993
Maneb	Hydrolysis with hydrochloric acid containing stannous chloride	Ion-exchange column, GC	0.1 mg/kg	No data	Newsome 1974 in IARC 1996

Table 6-2. Analytical Methods for Determining Manganese in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Wash water, tank mixes, cellulose acetate filters, gauze patches, clothing	Acid digestion in nitric acid or nitric acid-H_2O_2 mixture	ICP-AES (measured as Mn)	0.020mg/L (based on 0.004 mg Mn/L) in wash water and tank mixes; 0.5 µg (based on 0.1 µg Mn) in cellulose acetate filters, gauze patches, clothing	No data	Headley et al. 1996
Cucumber, tomato	Crush cucumber or tomato; extract by sonication with EDTA and methanol; 0.2% solution 2,6-dibromoquinone 4-chlorimide (DBQ) or 2,6-dichloroquinone 4-chlorimide (DCQ) added	Spectrophotometry	≤2ppm	Percent extraction: 95.5% (cucumber), 89.2% (tomato)	Walash et al. 1993
Formulations; Grains: sorghum, paddy, and wheat;	For formulations, dissolve Dithane M-45 in HNO_3; dilute with 3N HNO_3; add potassium periodate and phosphoric acid	AAS	0.6 µg to 6 µg Dithane M-45 (at 20g sample level);	95-100% (formulations)93-99% (paddy samples)	Rangaswamy and Vijayashankar 1975

[a]Percent recovery at manganese concentration greater than 80 µg/L; at lower concentrations (10–20 µg/L) percent recoveries were greater than 120%.

AAS = atomic absorption spectrometry; APDC = ammonium pyrrolidine dithiocarbamate; APHA = American Public Health Association; EPA = Environmental Protection Agency; FPD = flame photometric detection; ICP/AES = inductivity coupled plasma atomic emission spectroscopy; MED = microwave emission detector; MIBK = methyl isobutyl ketone; NIOSH = National Institute for Occupational Safety and Health; XRF = x-ray fluorescence

that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect

Exposure. Sensitive and selective methods are available for the detection and quantitative measurement of manganese in blood, urine, hair, feces, and tissues (Baselt 1988; Collipp et al. 1983; Friedman et al. 1987; Kneip and Crable 1988a; NIOSH 1984c, 1984d). Since levels in biological samples are generally rather low, sample contamination with exogenous manganese can sometimes occur (Tsalev 1983; Versieck et al. 1988). Development of standard methods for limiting this problem would be useful. As discussed in Section 2.5.1, measurement of average manganese concentrations in these materials has proved useful in comparing groups of occupationally exposed people to nonexposed people (Roels et al. 1987b) but has not been especially valuable in evaluating human exposure in individuals (Rehnberg et al. 1982). This is due to the inherent variability in intake levels and toxicokinetics of manganese in humans, rather than a limitation in the analytical methods for manganese. Development of noninvasive methods for measuring whole-body or tissue-specific manganese burdens would be valuable in estimating human exposure levels but would be limited by the same considerations of individual variability that limit existing methods.

Effect. No reliable biomarkers of manganese effect are known. Biochemical changes such as altered blood or urinary levels of steroids, neurotransmitters, or their metabolites are plausible biomarkers of exposure, but this possibility has not been thoroughly investigated. Although methods exist for the analysis of these biochemicals, further work to improve the analyses does not seem warranted unless the utility of this approach is established.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. All humans are exposed to manganese, primarily through food (EPA 1984a). Near a hazardous waste site that contains manganese or a factory that uses manganese, humans could receive above-average exposure by inhalation of air or ingestion of water, soil, or food. Methods exist for the analysis of manganese in all of these media, and the sensitivity of these methods is sufficient to detect levels of potential human health concern (APHA 1985a, 1985b, 1985c, 1985d, 1985e; EPA 1982, 1986b, 1986c; NIOSH 1984a, 1984b). However, there is a data need for analytical methods that can differentiate

between the differing manganese species in various environmental media. This information will add to our knowledge of whether a specific manganese species present at a waste site is cause for concern.

6.3.2 Ongoing Studies

No information was located regarding ongoing research on methods for analysis of manganese in biological materials or environmental samples.